

In the Specification

Please replace page 1, first paragraph with the following paragraph:

--This application is a continuation of application Serial No. 10/453,176, filed June 2, 2003, which is a continuation of application Serial No. 10/359,934, filed February 7, 2003, which is a continuation of application Serial No. 10/327,541, filed December 20, 2002, which is a continuation of application Serial No. 10/265,451, filed October 3, 2002, which is a continuation of application Serial No. 09/736,630, filed December 13, 2000, which is a continuation of application Serial No. 09/364,039 (now U.S. Patent No. 6,416,757), filed July 30, 1999, which is a continuation of application Serial No. 08/823,893, filed March 17, 1997 (now U.S. Patent No. 5,959,087), which is a continuation of application Serial No. 08/344,133, filed November 23, 1994 (now U.S. Patent No. 5,644,034), which is a continuation-in-part of application Serial No. 07/828,956, filed February 18, 1992 (now abandoned), which is a national phase filing of international application PCT/AU90/00337, filed August 7, 1990, published in English on February 21, 1991, which claims the benefit of Australian applications AU PJ5662, filed August 7, 1989, and AU PJ7576, filed November 24, 1989, the disclosure disclosures of which is are incorporated herein by cross-reference reference in their entirety.--

Replacement heading for page 1, line 5 (double underline indicates insertion and strikethrough indicates deletion):

--~~Field of the Invention~~ FIELD OF THE INVENTION--

Replacement heading for page 1, line 17 (double underline indicates insertion and strikethrough indicates deletion):

--~~Background of the invention~~ BACKGROUND OF THE INVENTION--

Replacement heading for page 3, line 30, (double underline indicates insertion and strikethrough indicates deletion):

--SUMMARY OF THE PRESENT INVENTION--

Replacement paragraph for paragraph page 4, line 27 through page 5, line 3:

--In a second aspect the present invention consists in a ligand capable of binding to human TNF, the ligand being characterized in that when it binds to TNF the induction of endothelial procoagulant, tumour regression, induction of tumour fibrin deposition, cytotoxicity and receptor binding activities of the TNF are inhibited, the ligand binding to the TNF such that the epitope of the TNF defined by the topographic regions of residues 1-18 (Val₁-Arg₂-Ser₃-Ser₄-Ser₅-Arg₆-Thr₇-Pro₈-Ser₉-Asp₁₀-Lys₁₁-Pro₁₂-Val₁₃-Ala₁₄-His₁₅-Val₁₆-Val₁₇-Ala₁₈), 58-65 (Ile₅₈-Tyr₅₉-Ser₆₀-Gln₆₁-Val₆₂-Leu₆₃-Phe₆₄-Lys₆₅), 115-125 (Tyr₁₁₅-Glu₁₁₆-Pro₁₁₇-Ile₁₁₈-Tyr₁₁₉-Leu₁₂₀-Gly₁₂₁-Gly₁₂₂-Val₁₂₃-Phe₁₂₄-Gln₁₂₅) and 138-149 (Arg₁₃₈-Pro₁₃₉-Asp₁₄₀-Tyr₁₄₁-Leu₁₄₂-Asp₁₄₃-Phe₁₄₄-Ala₁₄₅-Glu₁₄₆-Ser₁₄₇-Gly₁₄₈-Gln₁₄₉), or the topographic region of residues 1-18 (Val₁-Arg₂-Ser₃-Ser₄-Ser₅-Arg₆-Thr₇-Pro₈-Ser₉-Asp₁₀-Lys₁₁-Pro₁₂-Val₁₃-Ala₁₄-His₁₅-Val₁₆-Val₁₇-Ala₁₈), 108-128 (Gly₁₀₈-Ala₁₀₉-Glu₁₁₀-Ala₁₁₁-Lys₁₁₂-Pro₁₁₃-Trp₁₁₄-Tyr₁₁₅-Glu₁₁₆-Pro₁₁₇-Ile₁₁₈-Tyr₁₁₉-Leu₁₂₀-Gly₁₂₁-Gly₁₂₂-Val₁₂₃-Phe₁₂₄-Gln₁₂₅-Leu₁₂₆-Glu₁₂₇-Lys₁₂₈), or the topographic region of residues 56-79 (Tyr₅₆-Leu₅₇-Ile₅₈-Tyr₅₉-Ser₆₀-Gln₆₁-Val₆₂-Leu₆₃-Phe₆₄-Lys₆₅-Gly₆₆-Gln₆₇-Gly₆₈-Cys₆₉-Pro₇₀-Ser₇₁-Thr₇₂-His₇₃-Val₇₄-Leu₇₅-Leu₇₆-Thr₇₇-His₇₈-Thr₇₉), 110-127 (Glu₁₁₀-Ala₁₁₁-Lys₁₁₂-Pro₁₁₃-Trp₁₁₄-Tyr₁₁₅-Glu₁₁₆-Pro₁₁₇-Ile₁₁₈-Tyr₁₁₉-Leu₁₂₀-Gly₁₂₁-Gly₁₂₂-Val₁₂₃-Phe₁₂₄-Gln₁₂₅-Leu₁₂₆-Glu₁₂₇) and 135-155 (Glu₁₃₅-Ile₁₃₆-Asn₁₃₇-Arg₁₃₈-Pro₁₃₉-Asp₁₄₀-Tyr₁₄₁-Leu₁₄₂-Asp₁₄₃-Phe₁₄₄-Ala₁₄₅-Glu₁₄₆-Ser₁₄₇-Gly₁₄₈-Gln₁₄₉-Val₁₅₀-Tyr₁₅₁-Phe₁₅₂-Gly₁₅₃-Ile₁₅₄-Ile₁₅₅) is substantially prevented from binding to naturally occurring biologically active ligands.--

Replacement paragraph for the paragraph on page 5, lines 4-10:

--In a third aspect the present invention consists in a ligand which binds to human TNF in at least two regions selected from the group consisting predominantly of the topographic region of residues 1-20 (Val₁-Arg₂-Ser₃-Ser₄-Ser₅-Arg₆-Thr₇-Pro₈-Ser₉-Asp₁₀-Lys₁₁-Pro₁₂-Val₁₃-Ala₁₄-His₁₅-Val₁₆-Val₁₇-Ala₁₈-Asn₁₉-Pro₂₀), the topographic region of residues 56-77 (Tyr₅₆-Leu₅₇-Ile₅₈-Tyr₅₉-Ser₆₀-Gln₆₁-Val₆₂-Leu₆₃-Phe₆₄-Lys₆₅-Gly₆₆-Gln₆₇-Gly₆₈-Cys₆₉-Pro₇₀-Ser₇₁-Thr₇₂-His₇₃-Val₇₄-Leu₇₅-Leu₇₆-Thr₇₇), the topographic region of residues 108-127 (Gly₁₀₈-Ala₁₀₉-Glu₁₁₀-Ala₁₁₁-Lys₁₁₂-Pro₁₁₃-Trp₁₁₄-Tyr₁₁₅-Glu₁₁₆-Pro₁₁₇-Ile₁₁₈-Tyr₁₁₉-Leu₁₂₀-Gly₁₂₁-Gly₁₂₂-Val₁₂₃-Phe₁₂₄-Gln₁₂₅-Leu₁₂₆-Glu₁₂₇) and the topographic region of residues 138-149 (Arg₁₃₈-Pro₁₃₉-Asp₁₄₀-Tyr₁₄₁-Leu₁₄₂-Asp₁₄₃-Phe₁₄₄-Ala₁₄₅-Glu₁₄₆-Ser₁₄₇-Gly₁₄₈-Gln₁₄₉).--

Replacement paragraph for the paragraph beginning on page 5, line 26 and continuing on page 6, line 4:

--In a particularly preferred embodiment of the first, second and third aspects of the present invention the ligand is a monoclonal antibody selected from the group consisting of the monoclonal antibodies designated MAb 1, MAb 47 and MAb 54. Samples of the hybridoma cell lines which produce MAb 1, MAb 54 and MAb 47 have been deposited with the European Collection of Animal Cell Cultures (ECACC), Vaccine Research and Production Laboratory, Public Health Laboratory Service, Centre for Applied Microbiology and Research, Porton Down, Salisbury, Wiltshire SP4 OJG, United Kingdom. MAb 1 was deposited on 3 August 1989 and accorded accession No. 89080301; MAb 54 was deposited on 31 August 1989 and accorded accession No. 89083103; MAb 47 was deposited on 14 December 1989 and accorded accession No. 89121402, under the terms and conditions of the Budapest Treaty for the Deposit of Microorganisms for Patent purposes.--

Please replace the paragraph on page 7, lines 11-20, with the following paragraph:

--In a preferred embodiment of the sixth, seventh and eighth aspects of the present invention the ligand is the monoclonal antibody designated MAb 32. A sample of the hybridoma producing MAb 32 was deposited with The European Collection of Animal Cell Cultures (ECACC), Vaccine Research and Production Laboratory, Public Health Laboratory Service, Centre for Applied Microbiology and Research, Porton Down, Salisbury, Wiltshire SP4 OJG, United Kingdom on 3 August 1989 and was accorded accession No. 89080302, under the terms and conditions of the Budapest Treaty for the Deposit of Microorganisms for Patent purposes.--

Replacement paragraph for page 8, line 34 through page 9, line 9:

--In a sixteenth aspect the present invention consists in a ligand capable of binding to human TNF, the ligand being characterized in that when it binds to TNF the cytotoxicity and tumour regression ~~activities~~ activities of the TNF are unaffected; the induction of endothelial procoagulant and induction of tumour fibrin deposition activities of the TNF are inhibited and the tumour receptor binding activities of the TNF are unaffected, the ligand binding to TNF such that the epitope of the TNF defined by the topographic regions of residues 22-40 (Ala₂₂-Glu₂₃-Gly₂₄-Gln₂₅-Leu₂₆-Gln₂₇-Trp₂₈-Leu₂₉-Asn₃₀-Arg₃₁-Arg₃₂-Ala₃₃-Asn₃₄-Ala₃₅-Leu₃₆-Leu₃₇-Ala₃₈-Asn₃₉-Gly₄₀), 49-96 (Val₄₉-Val₅₀-Pro₅₁-Ser₅₂-Glu₅₃-Gly₅₄-Leu₅₅-Tyr₅₆-Leu₅₇-Ile₅₈-Tyr₅₉-Ser₆₀-Gln₆₁-Val₆₂-Leu₆₃-Phe₆₄-Lys₆₅-Gly₆₆-Gln₆₇-Gly₆₈-Cys₆₉-Pro₇₀-Ser₇₁-Thr₇₂-His₇₃-Val₇₄-Leu₇₅-Leu₇₆-Thr₇₇-His₇₈-Thr₇₉-Ile₈₀-Ser₈₁-Arg₈₂-Ile₈₃-Ala₈₄-Val₈₅-Ser₈₆-Tyr₈₇-Gln₈₈-Thr₈₉-Lys₉₀-Val₉₁-Asn₉₂-Leu₉₃-Leu₉₄-Ser₉₅-Ala₉₆), 110-127 (Glu₁₁₀-Ala₁₁₁-Lys₁₁₂-Pro₁₁₃-Trp₁₁₄-Tyr₁₁₅-Glu₁₁₆-Pro₁₁₇-Ile₁₁₈-Tyr₁₁₉-Leu₁₂₀-Gly₁₂₁-Gly₁₂₂-Val₁₂₃-Phe₁₂₄-Gln₁₂₅-Leu₁₂₆-Glu₁₂₇), and 136-153 (Ile₁₃₆-Asn₁₃₇-Arg₁₃₈-Pro₁₃₉-Asp₁₄₀-Tyr₁₄₁-Leu₁₄₂-Asp₁₄₃-Phe₁₄₄-Ala₁₄₅-Glu₁₄₆-Ser₁₄₇-Gly₁₄₈-Gln₁₄₉-Val₁₅₀-Tyr₁₅₁-Phe₁₅₂-Gly₁₅₃) is substantially prevented from binding to naturally occurring biologically active ligands.--

Please replace the paragraph on page 9, lines 20-29, with the following paragraph:

--In a preferred embodiment of the fifteenth, sixteenth and seventeenth aspects of the present invention the ligand is the monoclonal antibody designated MAb 42. A sample of the hybridoma cell line producing MAb 42 was deposited with The European Collection of Animal Cell Cultures (ECACC), Vaccine Research and Production Laboratory, Public Health Laboratory Service, Centre for Applied Microbiology and Research, Porton Down, Salisbury, Wiltshire SP4 OJG, United Kingdom on 3 August 1989 and was accorded accession No. 89080304, under the terms and conditions of the Budapest Treaty for the Deposit of Microorganisms for Patent purposes.--

Replacement paragraph for the paragraph beginning on page 11, line 10:

--In a twenty-fourth aspect the present invention consists in a ligand capable of binding to human TNF, the ligand being characterized in that when it binds to TNF the tumour fibrin deposition activity of the TNF is enhanced and the cytotoxicity, tumour regression, induction of endothelial procoagulant and tumour receptor binding activities of the TNF are inhibited, the ligand binding to the TNF such that the epitope of the TNF defined by the topographic regions of residues 1-20 (Val₁-Arg₂-Ser₃-Ser₄-Ser₅-Arg₆-Thr₇-Pro₈-Ser₉-Asp₁₀-Lys₁₁-Pro₁₂-Val₁₃-Ala₁₄-His₁₅-Val₁₆-Val₁₇-Ala₁₈-Asn₁₉-Pro₂₀) and 76-90 (Leu₇₆-Thr₇₇-His₇₈-Thr₇₉-Ile₈₀-Ser₈₁-Arg₈₂-Ile₈₃-Ala₈₄-Val₈₅-Ser₈₆-Tyr₈₇-Gln₈₈-Thr₈₉-Lys₉₀) is substantially prevented from binding to naturally occurring biologically active ligands.--

Replacement paragraph for page 11, line 26:

--In a preferred embodiment of the twenty-fifth aspect of the present invention the ligand binds to TNF in the topographic regions of residues 1-18 (Val₁-Arg₂-Ser₃-Ser₄-Ser₅-Arg₆-Thr₇-Pro₈-Ser₉-Asp₁₀-Lys₁₁-Pro₁₂-Val₁₃-Ala₁₄-His₁₅-Val₁₆-Val₁₇-Ala₁₈) and 76-90 (Leu₇₆-Thr₇₇-His₇₈-Thr₇₉-Ile₈₀-Ser₈₁-Arg₈₂-Ile₈₃-Ala₈₄-Val₈₅-Ser₈₆-Tyr₈₇-Gln₈₈-Thr₈₉-Lys₉₀).--

Replacement paragraph for page 11, line 29:

--In a preferred embodiment of the twenty-third, twenty-fourth and twenty-fifth aspects of the present invention the ligand is the monoclonal antibody designated MAb 21. A sample of the hybridoma cell line producing MAb 21 was deposited with the European Collection of Animal Cell Cultures (ECACC), Vaccine Research and Production Laboratory, Public Health Laboratory Service Centre for Applied Microbiology and Research, Porton Down, Salisbury, Wiltshire SP4 OJG, United Kingdom on 25 January 1990 and was accorded accession No. 90012432, under the terms and conditions of the Budapest Treaty for the Deposit of Microorganisms for Patent purposes.--

Please replace the paragraph on page 12, lines 10-20 with the following:

--In a twenty-seventh aspect the present invention consists in a ligand capable of binding to human TNF, the ligand being characterized in that when it binds to TNF the tumour fibring deposition activity of the TNF is unaffected and the cytotoxicity, tumour regression, induction of endothelial procoagulant and receptor binding activities of the TNF are inhibited, the ligand binding to the TNF such that the epitope of the TNF defined by the topographic regions of residues 22-40 (Ala₂₂-Glu₂₃-Gly₂₄-Gln₂₅-Leu₂₆-Gln₂₇-Trp₂₈-Leu₂₉-Asn₃₀-Arg₃₁-Arg₃₂-Ala₃₃-Asn₃₄-Ala₃₅-Leu₃₆-Leu₃₇-Ala₃₈-Asn₃₉-Gly₄₀), 69-97 (Cys₆₉-Pro₇₀-Ser₇₁-Thr₇₂-His₇₃-Val₇₄-Leu₇₅-Leu₇₆-Thr₇₇-His₇₈-Thr₇₉-Ile₈₀-Ser₈₁-Arg₈₂-Ile₈₃-Ala₈₄-Val₈₅-Ser₈₆-Tyr₈₇-Gln₈₈-Thr₈₉-Lys₉₀-Val₉₁-Asn₉₂-Leu₉₃-Leu₉₄-Ser₉₅-Ala₉₆-Ile₉₇), 105-128 (Thr₁₀₅-Pro₁₀₆-Glu₁₀₇-Gly₁₀₈-Ala₁₀₉-Glu₁₁₀-Ala₁₁₁-Lys₁₁₂-Pro₁₁₃-Trp₁₁₄-Tyr₁₁₅-Glu₁₁₆-Pro₁₁₇-Ile₁₁₈-Tyr₁₁₉-Leu₁₂₀-Gly₁₂₁-Gly₁₂₂-Val₁₂₃-Phe₁₂₄-Gln₁₂₅-Leu₁₂₆-Glu₁₂₇-Lys₁₂₈) and 135-155 (Glu₁₃₅-Ile₁₃₆-Asn₁₃₇-Arg₁₃₈-Pro₁₃₉-Asp₁₄₀-Tyr₁₄₁-Leu₁₄₂-Asp₁₄₃-Phe₁₄₄-Ala₁₄₅-Glu₁₄₆-Ser₁₄₇-Gly₁₄₈-Gln₁₄₉-Val₁₅₀-Tyr₁₅₁-Phe₁₅₂-Gly₁₅₃-Ile₁₅₄-Ile₁₅₅) is substantially prevented from binding to naturally occurring biologically active ligands.--

Please replace the paragraph on page 12, lines 26-35, with the following paragraph:

--In a preferred embodiment of the twenty-sixth, twenty-seventh and twenty-eighth aspects of the present invention the ligand is the monoclonal antibody designated MAb 53. A sample of the hybridoma cell line producing MAb 53 was deposited with the European Collection of Animal Cell cultures (ECACC), Vaccine Research and Production Laboratory, Public Health Laboratory Service, Centre for Applied Microbiology and Research, Porton Down, Salisbury, Wiltshire SP4 OJG, United Kingdom on 25 January 1990 and was accorded accession No. 90012433, under the terms and conditions of the Budapest Treaty for the Deposit of Microorganisms for Patent purposes.--

Please replace the paragraph on page 15, lines 3-10 with the following paragraph:

--In a thirty-sixth aspect the present invention consists in a ligand capable of binding to human TNF, the ligand being characterised in that when it binds to TNF the induction of endothelial procoagulant activity of the TNF is inhibited, the ligand binding to TNF such that the epitope of the TNF defined by the topographical region of residues 108 – 128 (Gly₁₀₈-Ala₁₀₉-Glu₁₁₀-Ala₁₁₁-Lys₁₁₂-Pro₁₁₃-Trp₁₁₄-Tyr₁₁₅-Glu₁₁₆-Pro₁₁₇-Ile₁₁₈-Tyr₁₁₉-Leu₁₂₀-Gly₁₂₁-Gly₁₂₂-Val₁₂₃-Phe₁₂₄-Gln₁₂₅-Leu₁₂₆-Glu₁₂₇-Lys₁₂₈) is prevented from binding to naturally occurring biologically active ligands.--

Please replace the paragraph on page 15, lines 11-13 with the following paragraph:

--In a thirty-seventh aspect the present invention consists in a ligand which binds to human TNF in the topographical region of residues 108 - 128 (Gly₁₀₈-Ala₁₀₉-Glu₁₁₀-Ala₁₁₁-Lys₁₁₂-Pro₁₁₃-Trp₁₁₄-Tyr₁₁₅-Glu₁₁₆-Pro₁₁₇-Ile₁₁₈-Tyr₁₁₉-Leu₁₂₀-Gly₁₂₁-Gly₁₂₂-Val₁₂₃-Phe₁₂₄-Gln₁₂₅-Leu₁₂₆-Glu₁₂₇-Lys₁₂₈).--

Insertion for page 15, between lines 31 and 32 (where the underline is as it appears in the text):

--Brief Description of the Drawings--

Replacement paragraph for page 16, lines 10-12:

--~~Fig. 7 shows~~ Figs. 7a, 7b and 7c show incorporation of labeled fibrinogen into tumours of tumour-bearing mice and the effect of anti-TNF MAbs;--

Replacement paragraph for page 16, line 17-18:

--~~Fig. 10 shows~~ Figs. 10a and 10b show the enhancement of TNF regression activity by MAb 32 in two experiments;--

Replacement paragraph for page 16, lines 19-20:

--~~Fig. 11 shows~~ Figs. 11a and 11b show the enhancement of TNF-induced tumour regression by MAb 32 - dose response at day 1 and day 2;--

Replacement paragraph for page 17, lines 13-14:

--~~Fig. 21 shows~~ Figs. 21a, 21b and 21c show MAb 32 reactivity with overlapping peptides of 10 AA length; and--

Insertion for page 18, between lines 6 and 7 (where the underline is as it appears in the text):

--Description of the Preferred Embodiments--.

Replacement paragraph for the paragraph on page 19, lines 6-9:

--The comparative ~~specificities~~ specificities of the monoclonal antibodies were determined in competition assays using either immobilized antigen (LACT) or antibody (PACT) (Aston and Ivanyi, 1985, Pharmac. Therapeut. 27, 403).--

Replacement paragraph for page 25, lines 1-2:

--8. MAb 1 reduces the uptake of ¹²⁵I fibrinogen into tumours of mice treated with TNF (~~Fig. 7~~Figs. 7a-c).--

Replacement paragraph for page 25, line 35 and continuing to page 26 line 7:

--Monoclonal antibody 32 variably enhances TNF-induced tumour regression activity against WEHI-164 fibrosarcoma tumours implanted subcutaneously into BALB/c mice at a TNF dose of 10µg/day (see ~~Figs. 10 and 11~~Figs. 10 a-b and 11a-b). This feature is not common to all monoclonal antibodies directed against TNF (Fig. 9) but resides within the binding site specificity of MAb 32 (Fig. 8) which may allow greater receptor mediated uptake of TNF into tumour cells (see Table 4).—

Replacement paragraph for page 27, lines 1-6:

--Conversely, MAb 32 enhances the *in vivo* activation of coagulation within the tumour bed as shown by the incorporation of radiolabelled fibrinogen (~~Fig. 7~~Figs. 7a-c). This may be due to activation of monocytes/macrophage procoagulant and may provide further insight into the mechanism of TNF-induced tumour regression.--

Replacement paragraph for page 32, lines 3-5:(single underlines indicate text underlined in original specification, double underlines indicated added text)

--Peptide 309

H-His-Val-Leu-Leu-Thr-His-Thr-Ile-Ser-Arg-Ile-Ala-Val-Ser-~~Thr~~Tyr-Gln-Thr-Lys-Val-Asn-Leu-Leu-COOH (73-94)--

Replacement paragraph for page 32, lines 6-7:(single underlines indicate text underlined in original specification, double underlines indicated added text)

--Peptide 323

H-Thr-Ile-Ser-Arg-Ile-Ala-Val-Ser-[Thr]Tyr-Gln-Thr-[OH]COOH (79-89)--

Replacement paragraph for page 33, lines 17-20:

--Peptide 301, 302, 305 are cleaved ~~from~~ from the resin with 95% TFA and 5% ~~thionisole~~ thioanisole (1.5h) and purified on reverse phase C4 column, (Buffer A- 0.1% aqueous TFA, Buffer B 80% CAN 20% A).--

Replacement paragraph for page 33, line 35:

--Typical results of MAb ELISA using the 7 and 10 mers are shown in ~~Fig 24~~Figs. 21a-c. Together with the results of PACT assays using the sheep anti-peptide sera (shown in Table 6) the following regions of TNF contain the binding sites of the anti-TNF MAbs.--

Replacement paragraph for page 34, lines 33-36):

--Note 1: - indicates no competition, + indicates slight competition at high concentration of anti-peptide antisera (1/50), ++++ indicates ~~treng~~ strong competition by anti-peptide sera equal to that of the homologous MAb.--

Replacement paragraph for page 38, lines 4-26:

--The genes of the mouse MAb32 antibody (IgG2b, Kappa) were rescued by PCR essentially as described (~~Clackson *et al.*, 1991, *supra*~~, Clackson *et al.*, 1991, in "PCR: a practical approach, A Practical Approach" eds. ~~Mr Phenex *et al.*~~, M. J. McPherson *et al.*, IRL Press, Oxford, pp 187-214) using the primers VH1BACK and VH1FOR2 for the VH gene and Vk2BACK and VK4FOR for the VL gene and the polymerase chain reaction (PCR, R.K. Saiki *et al.*, 1985, Science 230, p1350). The mouse VH and Vk genes were assembled for expression as scFv fragments by PCR assembly (~~Clackson~~ Clackson *et al.*, *supra*) amplified with VH1BACKSfi and VFFOR4NOT and ligated into phagemid pHEN1 (H.R. Hoogenboom *et al.*, 1991 Nucl. Acids. Res. 19 pp4133-4137) as a SfiI-NotI cut restriction fragment, and electroporated into *E.coli* HB2151 cells. Of 96 clones analysed by ELISA (see below), 9 secreted TNF-binding soluble scFv fragments. Sequencing revealed in all clones a mouse VH of family IIB and a mouse Vk of family VI (E.A. Kabat *et al.*, 1991 Sequences of Proteins of Immunological Interest, US Public Health Services). Nucleotide mutations which were probably introduced by the PCR were detected by comparing the 9 sequences, and a clone with consensus sequence and binding activity (scFv-MAb32) chosen for further cloning experiments.—

Replacement paragraph for paragraph beginning on page 38, line 29 continuing to page 39, line 20:

--The murine V-genes were recloned for soluble expression of heavy (Fd, VHCH1) or light chain, by linking the mouse V-genes to the human CH1 (of the mu-isotype) or human Ck gene

respectively by splice overlap extension. The mouse Vk gene was amplified from scFv-MAb32 DNA with oligonucleotides MOJK1FORNX (binds in joining region of V-gene and MVKBASFI (binds in 5' region and adds SfiI restriction site); the human Ck was obtained by PCR from a mouse-human chimaeric light chain gene (of NQ10.12.5, described in Hoogenboom et al., 1991 *supra* supra), with oligonucleotides MOVK-HUCK-BACK (binds in 5' of human Ck and is partially complementary with mouse Jk 1 region) and HUCKNOT16NOMYC (sits in 3' end of human Ck, retains the terminal cysteine, and tags on a NotI restriction site) as in ~~Clarkson et al.~~, Clackson et al., 1991 using a two fragment assembly. For linkage of the DNA fragments, the two PCR fragments were mixed and amplified with MVKBASFI and HUCKNOT16NOMYC. The chimaeric VkCk gene was subsequently cloned as a SfiI-NotI fragment in pUC19 derivative containing the pelB signal peptide sequence and appropriate cloning sites for soluble expression of the light chain (pUC19-pelB-myc). Similarly, the mouse VH gene (amplified from scFv-MAb32 with LMB3 and VH1FOR-2) was combined by splicing by overlap extension PCR with the human u-CH1 domain (amplified from human IgM-derived cDNA (Marks et al., 1991, *supra* supra; WO 92/01047) with Mo-VH-Ku-CH1 and HCM1FONO, and cloned as SfiI-NotI fragment into a pUC19-pelB-myc for soluble expression of a tagged chain.—

Replacement paragraph for page 42, line 30 through page 43, line 9:

--Three selected V λ genes were recloned in pUC19-pelB-myc for soluble expression V λ C λ chains. *E.coli* cells harbouring the three light chain plasmids were mixed, infected with a phage library of human VHCH1 genes, expressed from the fd-tet-DOC1 library described earlier and the library subjected to rounds of panning on TNF-coated Immuno tubes. Clones were picked after 5 rounds, when the titre of eluted phage increased 100-fold. Fifteen out of 20 clones analysed by BstNI fingerprint of the DNA insert used one of two ~~pattens~~ patterns (with approximately the same frequency). The 15 clones when combining their heavy chain VHCH1 fragments with the V λ A2 light chain gave stronger phase ELISA signals than when combined with the V λ C4 or V λ D1 light chain. Background signals obtained with phage displaying the heavy chain VHCH1 fragment only were similar to the signal of the murine VH-human CH1.--